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(54) Title: PROTEIN POWDER FOR PULMONARY DELIVERY

(57) Abstract: This invention provides particles of a therapeutic protein core coated with a surfactant that are suitable for pulmonary delivery to the deep lung of a patient in need thereof. Spray drying processes for preparing powders of the core protein particles and powders of the respirable, surfactant-coated, therapeutic protein particles are provided.

PROTEIN POWDER FOR PULMONARY DELIVERY

This invention is in the field of human medicine. In particular, this invention is in the field of pulmonary delivery of powders comprising therapeutic protein particles that are useful for treating various diseases.

Administration of therapeutic proteins by inhalation is an attractive method of treating patients suffering from diseases compared to painful delivery by injection. Surfactants may be advantageously combined with therapeutic proteins to form particles with improved aerosolization, enhanced absorption, altered time action, reduced agglomeration and reduced phagocytosis.

Spray drying is a commonly employed method of efficiently producing particles of therapeutic agents in the respirable range, namely, 1 μm to 5 μm MMAD. For example, Edwards, D. A. *et al.*, in U.S. Patent No. 5,985,309, issued 16 November 1999, completely solubilized feed stock solutions containing insulin, the surfactant dipalmitoyl phosphatidylcholine (DPPC) and other excipients prior to spray drying. Aqueous ethanol solutions comprising 2% insulin and 60% DPPC by weight were spray dried to form respirable particles.

One problem with the known processes for making therapeutic protein particles comprising a surfactant for pulmonary delivery is the step of solubilizing all components in a starting solution. For the preparation of respirable particles comprising a therapeutic protein and a surfactant, which generally have contrasting solubility properties, this solubility step limits the concentration of the protein, the surfactant, or both, and thus limits their incorporation level and their relative proportion in the resulting particles. Therefore, the maximal benefits of both the therapeutic protein and the surfactant as noted

above may not be achievable in particles prepared by the known processes.

Also, in the known processes of preparing protein particles from a solution, all of the components, namely, the therapeutic protein, the surfactant and other excipients, are intermixed and may thus interact with one another within the interior of the respirable particle. Unfortunately, these interactions may lead to chemical degradation of the protein during particle formation as well as chemical and physical instability during subsequent storage.

Emulsions and microemulsions are also used to prepare dried therapeutic protein particles in combination with a surfactant. These procedures, however, also face the problem of limited flexibility in the proportion of protein and surfactant that may be employed, and often lead to low levels of protein incorporation in the particles. Since emulsions are thermodynamically unstable and intermix the therapeutic protein and the surfactant, forming protein particles from emulsions is also fraught with stability problems.

We have discovered protein particles, and processes for making them, wherein the incorporation levels and relative proportion of protein and surfactant within the particle can be varied over a much wider range than is possible with current processes, and most significantly, wherein the surfactant is primarily localized at the surface of the particle. Thus, the present invention solves the limitations of current protein particles as outlined above.

Accordingly, one aspect of the present invention is a particle suitable for pulmonary delivery to the deep lungs of patients in which a surfactant coats a therapeutic protein core. A powder suitable for pulmonary delivery that is made up of these particles represents another aspect of

the invention. The powder is useful for the treatment of various diseases and disorders in mammals.

Another aspect of the invention is a process for preparing a powder suitable for pulmonary delivery comprising surfactant-coated therapeutic protein particles. The process involves spray drying therapeutic protein particles that are suspended in a liquid carrier comprising a surfactant, then recovering the resulting powder.

Hormones, antibodies and enzymes are examples of therapeutic proteins that may be incorporated into the particles and powders of the present invention.

Insulin, insulin analogs, insulin derivatives, GLP-1, GLP-1 analogs and GLP-1 derivatives are particularly useful therapeutic proteins that may be incorporated into particles and powders suitable for pulmonary delivery according to this invention. Another aspect of the invention is the use of a powder comprising these therapeutic proteins to prepare a medicament for the treatment of diabetes or hyperglycemia by pulmonary administration. The invention also provides a method of treating diabetes or hyperglycemia by administering to a patient in need of such treatment an effective amount of a powder comprising these therapeutic proteins by inhalation into the deep lung of the patient.

As used in the present specification, the word "protein" refers to strands of amino acids connected by peptide bonds. Proteins may contain one or more strands of amino acids connected together by covalent bonds, such as disulfide bonds, or by non-covalent interactions. Small proteins, such as those comprising fewer than 50 amino acids, may also be referred to as "peptides", but are distinctly included within the definition of the term "protein" as used herein.

The term "therapeutic protein" refers to those proteins that have demonstrated biological activity and may be

employed to treat a disease or disorder by delivery to a patient in need thereof by an acceptable route of administration. The biological activity of therapeutic proteins may be demonstrated *in vitro* or *in vivo* and results from interaction of the protein with receptors and/or other intracellular or extracellular components leading to a biological effect.

Examples of therapeutic proteins include hormones, antibodies and enzymes. See, for example, Platz, R. M. *et al.*, in U.S. Patent No. 6,051,256, issued 18 April 2000, which includes in Table 1 a list of many therapeutic proteins and their indications.

As used herein, the word "hormone" refers to a protein having hormonal activity and includes naturally occurring hormones and analogs and derivatives thereof.

As used herein, protein hormones include, *inter alia*, colony stimulating factors, such as granulocyte colony stimulating factor and macrophage colony stimulating factor; poietins such as erythropoetin (EPO) and thrombopoietin; growth factors such as growth hormone releasing factor, epidermal growth factor, fibroblast growth factor, hepatocyte growth factor, insulin-like growth factors and nerve growth factor; growth hormones such as human growth hormone; interferons, such as interferon-alpha-2a, interferon-alpha-2b, interferon-beta-1a, interferon-beta-1b, interferon-alpha-n3 and gamma-interferon; interleukins, such as interleukin-1, interleukin-3, interleukin-4, interleukin-6, interleukin-10, interleukin-11 and interleukin-12; metabolic hormones such as insulin, glucagon-like peptide-1 (GLP-1), glucagon, leptin and amylin; soluble receptors such as interleukin-1 receptor; fertility hormones such as follicle stimulating hormone (FSH), leutinizing hormone and chorionic gonadotropin; stem cell factors; and miscellaneous hormones such as parathyroid hormone, GLP-2, exendin-3,

exendin-4, ciliary neurotrophic factor, alpha-1-antitrypsin and calcitonin.

As used herein, the word "antibodies" refers to structurally related glycoproteins which bind to antigens. Therapeutic antibodies include, *inter alia*, monoclonal antibodies, IgA, IgD, IgE, IgG and IgM isotype antibodies, humanized antibodies, human antibodies, chimeric antibodies, antibody conjugates, and fragments of antibodies that retain their ability to bind to an antigen, such as F_{ab} and the like.

Therapeutic enzymes include, *inter alia*, DNase, activated protein C, tissue plasminogen activator, and coagulation factors such as factor VIIa, factor IXa and factor Xa.

Therapeutic proteins incorporated in the particles and powders of the present invention may contain naturally occurring L-amino acids or unnatural amino acids, such as D-amino acids. The amino acid sequence of the proteins may be identical to those occurring naturally in animals or other organisms or may be analogs in which the sequence is altered in various ways. In analogs of proteins, one or more amino acids may be added, deleted or replaced by other amino acids at the N-terminal, C-terminal or internal portions of the protein. Analogs of proteins are well known in the art.

Therapeutic proteins incorporated in the particles and powders of the present invention may also be modified by attachment of organic chemical groups to one or more amino acid side chains, to the N-terminal amino group or to the C-terminal carboxyl group of the protein. Such modifications are referred to herein as "derivatives" of the proteins. Examples of protein derivatives include glycopeptides in which naturally occurring polysaccharides are attached to the side chains of the amino acids asparagine or threonine. Other derivatizing groups include polyethylene glycol,

thyroxyl, carbamyl, succinyl, acetyl, methyl, amide, phosphoryl and fatty acid acyl groups and the like. Derivatives of proteins are well known in the art.

A protein incorporated in the particles and powders of the present invention may be present in a variety of forms, including a pharmaceutically acceptable salt form. A pharmaceutically acceptable salt of a protein means a salt formed between any one or more of the charged groups in the protein and any one or more pharmaceutically acceptable, non-toxic cations or anions. Organic and inorganic salts include, for example, ammonium, sodium, potassium, Tris, calcium, zinc or magnesium and those prepared from acids such as hydrochloric, sulfuric, sulfonic, tartaric, fumaric, glycolic, citric, maleic, phosphoric, succinic, acetic, nitric, benzoic, ascorbic, p-toluenesulfonic, benzenesulfonic, naphthalenesulfonic, propionic, carbonic, and the like.

The therapeutic proteins incorporated in the particles and powders of the present invention may be prepared by chemical synthesis techniques including classical solution-phase methods, solid phase methods, semi-synthetic methods or other methods well known to those skilled in the art. The therapeutic proteins incorporated in the particles and powders of the present invention may also be prepared by biosynthesis using recombinant DNA technology. For example, see Chance, R. E., *et al.*, U.S. Patent No. 5,514,646, issued 7 May 1996; Chance, R. E., *et al.*, EPO publication number 383,472, 7 February 1996; Brange, J., *et al.*, EPO publication number 214,826, 18 March 1987; and Belagaje, R. M., *et al.*, U.S. Patent No. 5,304,473, issued 19 April 1994. Using rDNA technology, proteins or precursors thereof may be biosynthesized in any number of host cells including bacteria, mammalian cells, insect cells, yeast or fungi. Examples of biosynthesis in mammalian cells and transgenic

animals are described in Hakola, K. [Molecular and Cellular Endocrinology, 127:59-69, (1997)].

Therapeutic proteins incorporated in the particles and powders of the present invention include those produced by isolation and purification from tissues, glands, organs, blood, urine or any other component of animals. An example of a protein in this embodiment is pork insulin produced from the pancreas of pigs.

Without limiting the generality of the scope of the present invention, several specific therapeutic proteins and groups of proteins will be named to better instruct the reader.

A preferred group of proteins for incorporation in the particles and powders of the invention consists of hormones, antibodies and enzymes. A more preferred group of proteins consists of hormones. A preferred group of hormones consists of growth hormones, metabolic hormones and fertility hormones.

Another preferred group of therapeutic proteins for incorporation in the particles and powders of the invention consists of native forms of insulin including human insulin, pork insulin and beef insulin.

Another preferred group of proteins for incorporation in the particles and powders of the invention consists of monomeric insulin analogs. For example, see Balschmidt, P., *et al.*, U.S. Patent No. 5,164,366, issued 17 November 1992; Brange, J., *et al.*, U.S. Patent No. 5,618,913, issued 8 April 1997; Chance, R. E., *et al.*, U.S. Patent No. 5,514,646, issued 7 May 1996; and Ertl, J., *et al.*, U.S. Patent No. 6,221,633, issued 24 April 2001. Particularly preferred are those monomeric insulin analogs wherein the amino acid residue at position B28 is Asp, Lys, Ile, Leu, Val or Ala and the amino acid residue at position B29 is Lys or Pro. The most preferred monomeric insulin analogs are

Lys(B28)Pro(B29)-human insulin, Asp(B28)-human insulin, Lys(B3)Glu(B29)-human insulin and Lys(B3)Ile(B28)-human insulin.

Another preferred group of proteins for incorporation in the particles and powders of the present invention consists of insulin analogs wherein the isoelectric point of the insulin analog is between 7.0 and 8.0. These analogs are referred to as pI-shifted insulin analogs. A more preferred group of pI-shifted analogs consists of Arg(B31)Arg(B32)-human insulin and Gly(A21)Arg(B31)Arg(B32)-human insulin.

Another preferred group of proteins for incorporation in the particles and powders of the present invention consists of derivatives of insulins and derivatives of insulin analogs. A more preferred group of proteins for incorporation in the particles and powders of the present invention are acylated derivatives of insulins and acylated derivatives of insulin analogs. A more preferred group of proteins consists of acylated derivatives of insulin and acylated derivatives of insulin analogs wherein the acyl group consists of straight chain, saturated fatty acids. Examples of straight chain, saturated fatty acids include carbon lengths of C4, C6, C8, C10, C12, C14, C16 and C18. A most preferred group of proteins for incorporation in the particles and powders of the present invention consists of palmitoyl-ε-Lys(B29)-human insulin and myristoyl-ε-Lys(B29)-des(B30)-human insulin, wherein the palmitoyl(C16) and myristoyl(C14) straight chain fatty acids are attached to the epsilon (ε) amino group of the Lys(B29) residue. For example, see Hughes, *et al.*, U.S. Patent No. 6,051,551, issued 18 April 2000; Baker, J. C., *et al.*, U.S. Patent No. 5,922,675, issued 13 July 1999; and Havelund, S., *et al.*, U.S. Patent No. 5,750,497, issued 12 May 1998.

Another preferred group of proteins for incorporation in the particles and powders of the present invention consists of glucagon-like peptide-1 (GLP-1), GLP-1 analogs, derivatives of GLP-1 and derivatives of GLP-1 analogs. By custom in the art, the amino-terminus of GLP-1(7-37)OH has been assigned number 7 and the carboxyl-terminus has been assigned number 37. A more detailed description of GLP-1 analogs and derivatives is found in Hoffmann, J. A., WO 99/29336, published 17 June 1999; Nielson, J. et al., WO00/07617, published 17 February 2000; and Knudsen, L.B. et al., J. Med. Chem. 43:1664-1669 (2000). A more preferred group of proteins for incorporation in the particles and powders of the present invention consists of native GLP-1(7-36)NH2, native GLP-1(7-37)OH, Val(8)-GLP-1(7-37)OH, Gly(8)-GLP-1(7-37)OH and Arg(34)-N-ε-(γ-Glu(N-α-hexadecanoyl))-Lys(26)-GLP-1(7-37)OH.

Another preferred group of polypeptides for incorporation in the particles and powders of the present invention consists of exendin, exendin analogs, derivatives of exendin and derivatives of exendin analogs. Exendin proteins and analogs include exendin-3 and exendin-4, described by Young, A., *et al.* [WIPO publication WO00/41546, 20 July 2000]. Examples of derivatives of exendin and derivatives of exendin analogs are those described by Knudsen, *et al.* [WIPO publication WO99/43708, 2 September 1999]. A more preferred therapeutic protein for incorporation in the particles and powders of the present invention is exendin-4.

Another preferred group of proteins for incorporation in the particles and powders of the present invention consists of leptin, leptin analogs, derivatives of leptin and derivatives of leptin analogs. Another preferred group of proteins consists of glycosylated leptin analogs. A more detailed description of the sequence of native leptin and

examples of leptin analogs is found in Beals, J. M., *et al.*, EPO publication number 849,276, 24 June 1998.

Another preferred group of proteins for incorporation in the particles and powders of the present invention consists of the full length human parathyroid hormone PTH(1-84), fragments such as PTH(1-38), PTH(1-34), PTH(1-37) and PTH(1-41) and analogs and derivatives thereof [see Chang, C-M., *et al.*, WIPO publication WO 99/29337, 17 June 1999, and Patton, U.S. Patent No. 6,080,721, issued 27 June 2000]. A more preferred group of proteins consists of human PTH(1-34), human PTH(1-38) and human PTH(1-84).

Another preferred group of proteins for incorporation in the particles and powders of the present invention is recombinant FSH and recombinant variants, analogs and derivatives thereof. FSH is a heterodimeric glycoprotein in which the alpha and beta subunits bind non-covalently. A more detailed description is found in Hoffmann, J. A., *et al.*, WIPO publication WO 00/04913, 3 February 2000.

Another preferred group of proteins for incorporation in the particles and powders of the present invention consists of recombinant human growth hormone (HGH), recombinant bovine growth hormone (BGH) and analogs and derivatives thereof. A more preferred group of proteins consists of recombinant HGH and recombinant BGH.

Another preferred group of proteins for incorporation in the particles and powders of the present invention consists of erythropoetin (EPO), EPO analogs, EPO derivatives and derivatives of EPO analogs. A more preferred group of proteins consists of recombinant, glycosylated human EPO, whose sequence was described by Lin, K-F., in U.S. Patent No. 4,703,008, issued 27 October 1987, and analogs and derivatives thereof. See also Mehta, *et al.*, U.S. Patent No. 6,001,800, issued 14 December 1999.

The word "particle" refers to a small, discrete subdivision of solid material. Although there is no size limitation to this term, the protein particles as described herein will generally be in the range of 0.5 μm to 100 μm in diameter. The therapeutic protein particles of the present invention may be in an amorphous or crystalline form, and may alternatively comprise other excipients. Examples of excipients include salts such as sodium citrate, divalent metal ions such as zinc ions, and non-therapeutic proteins such as protamine and albumin. The word "powder" refers to a loose grouping or aggregation of dry particles.

The abbreviations "MMAD" and "MMEAD" are well-known in the art, and stand for "mass median aerodynamic diameter" and "mass median equivalent aerodynamic diameter", respectively. The terms are substantially equivalent. The "aerodynamic equivalent" size of a particle is the diameter of a unit density sphere which exhibits the same aerodynamic behavior of the particle, regardless of actual density or shape. MMAD is determined using a cascade impactor, which measures the particle size as a function of the aerodynamic behavior of the particle in a high velocity airstream. The mean (50%) particle size is obtained from a linear regression analysis of the cumulative distribution data.

Reference to a powder that is "suitable for pulmonary administration" means the particles that make up the powder have an MMAD of less than 10 μm, preferably 1 μm to 5 μm MMAD, and more preferably in the range of 1 μm to 4 μm MMAD, or from 1 μm to 3 μm MMAD, and, most preferably, from 2 μm to 3 μm MMAD.

For nonporous protein particles, measurement of the volume median diameter using a Coulter counter is also useful. For solid, nonporous protein particles, a volume median diameter suitable for pulmonary delivery is

preferably 1 μm to 5 μm, and more preferably in the range of 1 μm to 4 μm, or from 1 μm to 3 μm and, most preferably, from 2 μm to 3 μm.

The term "incorporation level" refers to the content, by weight, of a specified component in a particle or powder compared to the total weight of the particle or powder.

The term "pulmonary delivery" is generally equivalent to the term "pulmonary administration" and means introducing a therapeutic protein powder of the present invention into the deep lung of a patient in need thereof.

An aerosol comprising the therapeutic protein particles may be administered alone or in any appropriate pharmaceutically acceptable carrier, such as a liquid, for example saline, or as a powder, for administration to the deep lung. It can also be delivered with larger carrier particles, not including a therapeutic agent, having an MMAD in the range of 50 μm to 100 μm.

The proper aerosol dosage, formulation and delivery system for a powder of the present invention may be selected for a particular therapeutic application by techniques known to those skilled in the art. See, for example, Gonda, I., [Critical Reviews in Therapeutic Drug Carrier Systems, 6:273-313, 1990] and Moren [Aerosols in Medicine: Principles, Diagnosis and Therapy; Moren, *et al.*, Eds., Elsevier, Amsterdam, 1985].

There are many devices and methods known in the art that are useful for administering a therapeutic powder of the present invention by inhalation into the deep lungs of a patient in need of such treatment.

Included among the devices used to administer a therapeutic protein powder according to the present invention are, *inter alia*, metered dose inhalers, liquid nebulizers, dry powder inhalers, sprayers, thermal

vaporizers, and the like, and those provided by developing technology, including the AERx® pulmonary drug delivery system being developed by Aradigm Corporation, the dry powder and delivery devices being developed by Inhale Therapeutic Systems, Inc., and the Spiros® dry powder inhaler system being developed by Dura Pharmaceuticals, Inc. Other suitable technologies include electrohydrodynamic aerosolizers being developed at Battelle (see, for example, WIPO publication WO00/64590, 2 November 2000) and piezoelectric ultrasonic particle generators used in devices such as the AeroDose™ Inhalers developed by AeroGen, Inc. (Sunnyvale, CA, USA). For good respirability, the inhalation device should deliver aerodynamically small particles, *e.g.*, less than 10 μm MMAD, preferably 1 to 5 μm MMAD, more preferably in the range of 1 μm to 3 μm MMAD, and most preferably from 2 μm to 3 μm MMAD. In addition, the inhalation device must be practical, in the sense of being easy to use, small enough to carry conveniently, capable of providing multiple doses, and durable. Some specific examples of commercially available inhalation devices suitable for the practice of this invention are Turbohaler (Astra), Rotahaler (Glaxo), Diskus (Glaxo), the Ultravent nebulizer (Mallinckrodt), the Acorn II nebulizer (Marquest Medical Products), the Ventolin metered dose inhaler (Glaxo), the Spinhaler powder inhaler (Fisons), and the like. Both uncoated protein particles and coated protein particles can be advantageously delivered by a dry powder inhaler or a sprayer. There are several desirable features of a dry powder inhalation device for administering the powders of the present invention. For example, delivery by such inhalation devices is advantageously reliable, reproducible, and accurate.

The term "effective amount" refers to a quantity of therapeutic protein powder deemed adequate and proper to elicit a biological response in a patient in need thereof. The effective amount will be determined by the biological activity of the protein employed and the amount needed in a unit dosage form. Because the powders are dispersible, it is preferred that they be manufactured in a unit dosage form in a manner that allows for ready and convenient use by the consumer.

As those skilled in the art will recognize, the nature and quantity of the pharmaceutical composition, and the duration of administration of a single dose also depend on the type of inhalation device employed. For some aerosol delivery systems, such as nebulizers, the frequency of administration and length of time for which the system is activated will depend mainly on the concentration of therapeutic protein powders in the aerosol. For example, shorter periods of administration can be used at higher concentrations of the therapeutic protein powders in the nebulizer solution. Devices such as metered dose inhalers can produce higher aerosol concentrations, and can be operated for shorter periods to deliver the desired amount of the powders. Devices such as dry powder inhalers deliver active agent until a given charge of agent is expelled from the device. In this type of inhaler, the quantity of therapeutic protein particles in a given quantity of the powder determines the dose delivered in a single administration.

The word "suspension" refers to a composition in which most or all of the therapeutic protein is present in the form of distinct, insoluble, solid particles in a liquid carrier. The therapeutic protein particle in the suspensions do not contain surfactant within its interior, that is, the protein particles are not formed in the

presence of surfactant. For the present specification, neither emulsions nor microemulsions are encompassed within the definition of the word suspension.

The word "core" refers to the interior, solid therapeutic protein portion of a respirable particle.

The word "coating" refers to a surfactant layer or layers surrounding and completely or partially engulfing a therapeutic protein core particle. "Uncoated particle" refers to a protein particle to which no surfactant coating has been applied.

The term "liquid carrier" refers to a liquid capable of maintaining therapeutic protein particles in a suspended state and, in addition, possesses a volatility and a viscosity suitable for use as feed stock for a spray-drying device. Examples of liquid carriers include water and organic solvents such as ethanol, methanol, acetone, acetonitrile and methylene chloride.

A preferred group of liquid carriers used in the present invention consists of organic solvents. Preferably, the use of organic solvents in the absence of water reduces the extent of microbial contamination of the resulting spray dried protein particles. More preferably, the liquid carrier is a single organic solvent and not a mixture of organic solvents.

The word "solution" refers to a liquid composition in which the therapeutic protein and all excipients that are present in the composition are completely or almost completely dissolved.

The abbreviation "DPPC" refers to 1,2-. dipalmitoylphosphatidylcholine, CAS Registry Number 2644-64-6. This compound has numerous synonyms in the literature, including dipalmitoyllecithin, 1,2-dihexadecanoyl phosphatidylcholine, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine, and 1,2-dipalmitoylglycerol-3-

phosphorylcholine. DPPC may be obtained from Avanti Polar Lipids, Inc., Alabaster, AL, USA.

The abbreviation "DSPC" refers to 1,2-distearoylphosphatidylcholine. This compound has numerous synonyms in the literature, including, 1,2-dioctadecanoyl phosphatidylcholine, 1,2-distearoyl-sn-glycero-3-phosphocholine, and 1,2-distearoylglycerol-3-phosphorylcholine. DSPC may be obtained from Avanti Polar Lipids, Inc., Alabaster, AL, USA.

The word "surfactant" refers to any pharmaceutically acceptable agent that preferentially absorbs to an interface between two immiscible phases, such as the interface between a solid and a liquid, or a water/air interface. Surfactants generally possess a hydrophilic moiety and a lipophilic moiety, such that, upon absorbing to particles, they tend to present moieties to the external environment that do not attract similarly coated particles, thus reducing particle agglomeration. Surfactants may also promote biological absorption of a therapeutic or diagnostic agent and thus increase the bioavailability of the agent.

Preferred groups of pharmaceutically acceptable surfactants useful in the present invention consist of zwitterionic, (e.g. N-alkyl-N,N-dimethylammonio-1-propane-sulfonates), non-ionic (e.g. polyoxyethylene sorbitan monolaurate, or Tween 20), anionic (e.g. sodium caprylate), cationic (e.g. cetylpryidinium chloride) and polymeric surfactants (e.g. polyethyleneglycol) and phospholipids.

A more preferred group of pharmaceutically acceptable surfactants consists of phospholipids. Examples of phospholipids include phosphatidylcholines, phosphatidylethanolamines, phosphatidylglycerols, phosphatidylserines. A more preferred group of phospholipids consists of phosphatidylcholines. Preferred phosphatidylcholines are DPPC, DSPC and 1,2-

dimyristoylphosphatidylcholine. A most preferred surfactant is DPPC. Mixtures of surfactants may also be advantageously employed in the present invention.

According to the present invention, a surfactant or mixture of surfactants is coated onto a therapeutic protein particle after the core particle is formed. The coating process itself may occur instantly upon mixing or may, alternatively or in addition, occur during subsequent solvent evaporation or spray drying procedures. The surfactant coating may represent a relatively thin or thick layer. The thickness of the surfactant coating may also be variable. Preferably the surfactant completely coats or engulfs the therapeutic protein core particle. The surfactant-coated particles may be porous or nonporous, but for prolonged absorption, the coated particles are preferably nonporous. For prolonged absorption, the surfactant coating is preferably thick and completely engulfs the therapeutic protein core particle. The MMAD or median diameter of the surfactant-coated protein particles should be within preferred limits, as described above, for optimized pulmonary delivery. Preferably, the surfactant-coated particles will not be prone to agglomeration and the bulk powder comprising the particles will be free-flowing.

The term "spray drying" refers to the conventional processing operation used to produce dry particulate solids from a liquid composition. The steps involved in spray drying are atomization of the feed stock liquid composition, spray-air contact followed by evaporation of the liquid. A wide variety of devices useful for conducting the spray drying operation is known to those skilled in the art. Examples of spray drying equipment include the Büchi Model 190 labtop spray dryer (BUCHI Analytical, New Castle, Delaware, USA) and the PSD™ pharmaceutical spray dryer (Niro Inc., Columbia, Maryland, USA) coupled with a GORE-

TEX® membrane filter bag using Teflon® B fiberglass fabric (W. L. Gore & Associates, Newark, Delaware, USA). After evaporation of the liquid, the solid material may be recovered from the collection chamber by a variety of tools, such as a spatula. A collection of spray drying procedures and techniques is described in Mehta, *et al.*, U.S. Patent No. 6,001,800, issued 14 December 1999.

The term "organic solvent" refers to a liquid organic compound such as methanol, ethanol, acetonitrile, acetone, methylene chloride and n-propanol. Preferred organic solvents for the present invention are ethanol, methanol and acetonitrile and the most preferred organic solvent is ethanol. Other well known organic solvents may be used.

For the present invention, the organic solvent is preferably freely miscible with water. The term "freely miscible" is equivalent to the term "totally miscible", which, as used in the present specification, means the organic solvent is one that can be combined with water to form a single liquid phase. Examples of organic solvents that are freely miscible with water include methanol, ethanol, acetone and acetonitrile. Preferred water miscible organic solvents for use in the present invention are methanol, ethanol and acetonitrile and the most preferred water miscible organic solvent is ethanol.

One embodiment of the present invention is a process for preparing a powder, which comprises surfactant-coated therapeutic protein particles, that is suitable for pulmonary delivery to the deep lung of a patient in need thereof. Starting materials for this process include uncoated therapeutic protein particles, which may be prepared by many alternative methods, including micronization, milling, freeze drying, jet milling, microcrystallization, spray drying, and the like, that are known to those of skill in the art. Preferably, the

uncoated particles are prepared by spray drying and, most preferably, they are prepared by the process described below for spray drying a therapeutic protein solution.

To make uncoated therapeutic protein particles by spray drying, the therapeutic protein is first added to a liquid solution. Preferably, water is a component of the liquid solution. Excipients may be combined with the therapeutic protein in the liquid solution. For example, a buffering component, such as sodium citrate, may be added to provide pH stability. Other excipients such as sodium chloride and non-therapeutic proteins such as albumin and protamine may also be solubilized in the therapeutic protein solution. Other excipients for inclusion in the therapeutic protein solution include divalent metal ions such as Fe+2, Zn+2, Co+2 and Cd+2. A preferred divalent metal ion is Zn+2. If it is desirable to prolong the absorption of the therapeutic protein after delivery to the deep lung of a patient, a group of preferred excipients in the solution consists of protamine and Zn+2.

The therapeutic protein and all excipients in the solution are almost completely or, more preferably, completely dissolved in the liquid. For the present invention, surfactants are excluded as a component of the therapeutic protein solution. The solution is not an emulsion or a microemulsion.

The preferred pH of the therapeutic protein solution is very dependent upon the specific therapeutic protein and the excipients included in the composition. The composition pH may be adjusted to solubilize the protein and all of the excipients. A preferred pH range for the therapeutic protein solution is pH 1 to pH 9. Another preferred range is pH 2 to pH 6. Another preferred range is pH 3 to pH 7. Another preferred range is pH 3 to pH 5. More preferred ranges are pH 2 to pH 4, pH 4 to pH 6, and pH 6 to pH 8.

The next step in this process is spray drying the therapeutic protein solution. This may be conducted by any spray drying technique and spray drying equipment. These processes and devices are known to those skilled in the art. After spray drying, the dried protein particles that are produced are recovered from the collection area.

Preferably, the uncoated therapeutic protein particles that make up the powder prepared as described in the previous paragraphs will have an MMAD that is slightly smaller than those particles most suitable for pulmonary delivery. This is because the surfactant coating that will be applied to these particles further increases their diameters. Preferably, the uncoated particles will not agglomerate to the extent that they clog the nozzle of the spray drying apparatus in the subsequent spray drying process. Therefore, the uncoated therapeutic (core) protein particles will generally have an MMAD of less than 6 μm, preferably 0.5 μm to 4 μm MMAD, more preferably in the range of 0.5 μm to 3 μm MMAD, and most preferably in the range of 1 μm to 2.5 μm MMAD.

For these nonporous protein (core) particles, measurement of the median diameter using a Coulter counter is also useful and the median diameter is preferably 0.5 μm to 4 μm, more preferably in the range of 0.5 μm to 3 μm, and most preferably in the range of 1 μm to 2.5 μm.

Therapeutic protein particles are used to prepare a suspension composition by combining them with a liquid carrier and a surfactant. The liquid carrier must be carefully selected to ensure the particles remain undissolved and in suspension. If water is a part of the liquid carrier, the pH and/or ionic strength of the liquid carrier may be adjusted to maintain the particles in suspension. Preferably, the liquid carrier is an organic

solvent or a mixture of organic solvents. More preferably, the organic solvent is freely miscible with water. More preferably, the organic solvent is ethanol, methanol, acetone or acetonitrile. Most preferably, the organic solvent is ethanol. The use of a neat organic solvent or solvents as the liquid carrier has the advantage of minimizing the risk of microbial growth in the resulting therapeutic protein powder. Suspension of a therapeutic protein particle in a neat organic solvent does not generally inactivate the protein.

Aggregates of the therapeutic protein particles suspended in the liquid carrier may be further dispersed by agitating the suspension, for example, by shaking, vortexing or gently sonicating the suspension. Preferably, coarse aggregates of the particles will be sufficiently dispersed that they will not clog the nozzle of the spray drying apparatus in the subsequent spray drying process.

The next step in the process for preparing surfactant-coated therapeutic protein particles is removal of the liquid carrier from the composition by solvent evaporation, which is preferably accomplished by spray drying. When spray drying is employed, the feed stock for the spray dryer is a suspension of therapeutic protein particles in a liquid carrier comprising a surfactant. Process parameters in spray drying operations are generally dependent upon the equipment being employed. Preferably, a flow rate is selected that is fast enough to prevent the nozzle from becoming clogged but not so fast that incomplete drying occurs. Preferably, an air atomization pressure is selected that will lead to formation of particles having an appropriate aerodynamic size and size distribution suitable for pulmonary delivery. Preferably, the inlet and outlet temperatures will be low enough that no or very little degradation of the protein or the particles will occur.

Preferably, the inlet temperature will be the range of 30°C to 100°C. Another preferred inlet temperature range is 50°C to 90°C. Another preferred inlet temperature range is 60°C to 80°C. Preferably, the outlet temperature will be in the range of 25°C to 80°C. Another preferred outlet temperature range is 35°C to 70°C. Another preferred outlet temperature range is 45°C to 60°C.

The suspension composition also includes a surfactant. More preferably, the surfactant is soluble in the liquid carrier. Most preferably, the surfactant is DPPC which is dissolved in ethanol. The range of ratios of surfactant to therapeutic protein, on a weight basis, in the suspension composition is preferably 1:99 to 99:1. Another preferred range of ratios of surfactant to therapeutic protein, on a weight basis, is 1:20 to 20:1. Another preferred range of ratios of surfactant to therapeutic protein, on a weight basis, is 1:5 to 5:1. Other preferred ranges of ratios of surfactant to therapeutic protein, on a weight basis, are 1:4 to 4:1, 1:3 to 3:1, 1:2 to 2:1, 20:1 to 99:1, 1:99 to 1:20, 4:1 to 20:1 and 1:20 to 1:4. More preferred ratios are 50:50, 10:90 and 90:10.

After spray drying, the dried powder that is produced is recovered from the collection area of the spray drying device. The incorporation level of therapeutic protein in the surfactant-coated particles, on a weight basis, is preferably 3% to 98%. More preferably the incorporation level is 4% to 90%. Other preferred ranges of incorporation levels of therapeutic protein in the coated particles are 4% to 20%, 5% to 45%, 20% to 50%, 50% to 80%, and 35% to 75%. Preferably, the surfactant-coated particles have an MMAD of less than 10 μm, preferably 1 μm to 5 μm MMAD, and more

preferably in the range of 1 μm to 4 μm MMAD, or from 1 μm to 3 μm MMAD, and, most preferably, from 2 μm to 3 μm MMAD.

Another embodiment of the present invention is a therapeutic protein powder, suitable for pulmonary delivery, prepared by the process described above. For this embodiment, the therapeutic protein is preferably selected from the group consisting of hormones, antibodies and enzymes, and, more preferably, the therapeutic protein is a hormone. Preferred hormones include native forms of insulin and analogs and derivatives thereof, GLP-1 and analogs and derivatives thereof, and more preferably, the therapeutic protein is human insulin or Val(8)-GLP-1(7-37)OH.

Another embodiment of the present invention is the use of the powder described in the previous paragraph to prepare a medicament for the treatment of patients afflicted with a disease or disorder and in need of such treatment by pulmonary administration. Powders containing insulin, an insulin analog, an insulin derivative, GLP-1, a GLP-1 analog or a derivative thereof may be employed to treat patients afflicted with diabetes or hyperglycemia by pulmonary administration. Such medicaments may contain other excipients in addition to the therapeutic protein powder. Such excipients include a liquid or solid carrier, a bulking agent, a preservative such as m-cresol, phenol and the like, an isotonicity agent such as glycerol, sodium chloride and the like, a non-therapeutic protein such as protamine, albumin, and the like, absorption enhancers such as bile salts, salts of fatty acids, phospholipids and the like, and other compounds that are thought to be safe for the patients being treated.

Another embodiment of the present invention is a powder prepared by the process described above that is suitable for pulmonary delivery to the deep lung of a patient in need of such treatment. For the treatment of diabetes or

hyperglycemia, the therapeutic protein incorporated into the particles of the powder is preferably a native form of insulin, an insulin analog, an insulin derivative, GLP-1, a GLP-1 analog or a derivative thereof. More preferably, the therapeutic protein is human insulin or Val(8)-GLP-1(7-37)OH.

Another embodiment of the present invention is a method of treating a disease or disorder comprising, administering to a patient in need thereof, an effective amount of a powder prepared by the process described above for preparing powders suitable for pulmonary administration. An effective amount of a powder may be determined by the potency of the therapeutic protein, the concentration of the therapeutic protein in the powder, the level of absorption obtained by pulmonary administration, the type and severity of the disease or disorder being treated, the pharmacodynamic and pharmacokinetic results generally achieved by pulmonary administration of the therapeutic protein, and other factors well understood by those skilled in the art. The effective amount of the powder may be administered to a patient in need thereof in a single delivery or breath, or by multiple delivery or breathing regimens.

Another embodiment of the present invention is a method of treating diabetes or hyperglycemia by administering to a patient in need thereof an effective amount of a powder prepared by a process described above for preparing powders suitable for pulmonary administration. For this embodiment, the therapeutic protein in the powder is preferably a native form of insulin, an insulin analog, an insulin derivative, GLP-1, a GLP-1 analog or a derivative thereof. More preferably, the therapeutic protein is human insulin or Val(8)-GLP-1(7-37)OH.

Another embodiment of the present invention is a powder suitable for pulmonary delivery to the deep lung of a

patient in need thereof, comprising therapeutic protein particles coated with a surfactant. The surfactant-coated therapeutic particles may be used in a therapy or therapeutic regimen to treat a patient in need thereof.

Another embodiment of the invention is the coated therapeutic protein particles themselves, which may be in an amorphous or crystalline form. Preferably, the surfactant-coated particles have an MMAD of less than 10 μm, preferably 1 μm to 5 μm MMAD, and more preferably in the range of 1 μm to 4 μm MMAD, or from 1 μm to 3 μm MMAD, and, most preferably, from 2 μm to 3 μm MMAD.

The particles comprising the powder of the present invention may be any shape, including spherical, oblong, irregular, jagged, and the like. Preferably, the particles are irregular or spherical in shape. The particles comprising the powder according of this embodiment may be porous or nonporous. Preferably, the particles are nonporous.

The particles of this powder comprise a core that includes the therapeutic protein and may also include various excipients, such as citrate, zinc ions and protamine, but does not include surfactant. Surfactant, however, provides the coating covering the exterior of the core protein particle. Examples 2-4, 8, 11 and 13 describe procedures to make powders according to this embodiment.

Preferably, the surfactant used as the coating of the particles in the powders of this embodiment of the invention is a phospholipid. More preferably, the surfactant is the phospholipid DPPC. The range of ratios of surfactant to therapeutic protein, on a weight basis, in the coated particles is preferably 1:99 to 99:1. Another preferred range of ratios of surfactant to therapeutic protein, on a weight basis, is 1:20 to 20:1. Another preferred range of

ratios of surfactant to therapeutic protein, on a weight basis, is 1:5 to 5:1. Other preferred ranges of ratios of surfactant to therapeutic protein, on a weight basis, are 1:4 to 4:1, 1:3 to 3:1, 1:2 to 2:1, 20:1 to 99:1, 1:99 to 1:20, 4:1 to 20:1 and 1:20 to 1:4. More preferred ratios are 50:50, 10:90 and 90:10.

The incorporation level of therapeutic protein in the surfactant-coated particles, on a weight basis, is preferably 3% to 98%. More preferably the incorporation level is 4% to 90%. Other preferred ranges of incorporation levels of therapeutic protein in the coated particles are 4% to 20%, 5% to 45%, 20% to 50%, 50% to 80%, and 35% to 75%.

Another embodiment of the present invention is the use of a powder comprising a therapeutic protein particle coated with a surfactant to prepare a medicament for the treatment by pulmonary administration of patients afflicted with a disease or disorder and in need of such treatment.

Another embodiment of the present invention is the use of a powder comprising a therapeutic protein particle coated with a surfactant to prepare a medicament for the treatment of diabetes or hyperglycemia by pulmonary administration. For this embodiment, the therapeutic protein is preferably a native form of insulin, an insulin analog, an insulin derivative, GLP-1, a GLP-1 analog or a derivative thereof. More preferably, the therapeutic protein is human insulin or Val(8)-GLP-1(7-37)OH. Such medicaments may comprise other excipients in addition to the therapeutic protein powder, such as a liquid or solid carrier, a bulking agent, a preservative such as m-cresol, phenol and the like, a divalent metal ion such as Zn+2, an isotonicity agent such as glycerol, sodium chloride and the like, a non-therapeutic protein such as protamine, albumin, and the like, absorption enhancers such as bile salts, salts of fatty acids,

phospholipids and the like, and other compounds that are thought to be safe to the patients being treated.

Another embodiment of the present invention is a method of treating a disease or disorder comprising, administering to a patient in need thereof, an effective amount of a powder suitable for pulmonary delivery, comprising a therapeutic protein particle coated with a surfactant. An effective amount of a powder may be determined by the potency of the therapeutic protein, the concentration of the therapeutic protein in the powder, the level of absorption obtained by pulmonary administration, the type and severity of the disease or disorder being treated, the pharmacodynamic and pharmacokinetic results generally achieved by pulmonary administration of the therapeutic protein, and other factors well understood by those skilled in the art. The effective amount of the powder may be administered to a patient in need thereof in a single delivery or breath, or by multiple delivery or breathing regimens.

Another embodiment of the present invention is a method of treating diabetes or hyperglycemia by administering to a patient in need thereof an effective amount of a powder, suitable for pulmonary delivery, comprising a therapeutic protein particle coated with a surfactant. For this embodiment, the therapeutic protein incorporated into the particles of the powder is preferably a native form of insulin, an insulin analog, an insulin derivative, GLP-1, a GLP-1 analog or a derivative thereof. More preferably, the therapeutic protein is human insulin or Val(8)-GLP-1(7-37)OH. An effective amount of a powder may be determined by the potency of the therapeutic protein, the concentration of the therapeutic protein in the powder, the level of absorption obtained by pulmonary administration, the type and severity of the disease being treated, the

pharmacodynamic and pharmacokinetic results generally achieved by pulmonary administration of the therapeutic protein, and other factors well understood by those skilled in the art. The effective amount of the powder may be administered to a patient in need thereof in a single delivery or breath or by multiple delivery or breathing regimens.

The present invention may be better understood with reference to the following examples. These examples are intended to be representative of specific embodiments of the invention and are not intended as limiting the scope of the invention.

Example 1

Preparation of Spray-dried Insulin Solid

Zinc crystals of human insulin were dissolved at 50 mg/mL in a solution of 0.1 M sodium citrate pH 2.0 buffer and filtered through a 0.45 μm filter. Using a Büchi 190 labtop spray dryer with an inlet temperature set at 81°C and outlet temperature at 49°C, the insulin solution was spray dried. The flow rate was about 5.7 mL/min and the air atomization pressure was about 32 psi. The powder of insulin particles was scraped from the glass collection jar with a spatula resulting in a yield of 79.4% by weight. The insulin particles had an MMAD of 2.08 μm as determined by using a cascade impactor.

Example 2

Preparation of Spray-dried Insulin:DPPC (50:50) Powder

A solution of DPPC (Avanti Polar Lipids, Inc., Alabaster, AL, USA) was prepared at a concentration of 25 mg/mL in absolute ethanol. A powder of insulin particles prepared as described in Example 1 was added to the

DPPC/ethanol solution at a concentration of 25 mg/mL. The insulin particles were further dispersed into the suspension by gentle sonication (Ultrasonic bath Model 8210, Branson Ultrasonics Corporation, Danbury, CT, USA). While being gently agitated, this suspension was then spray dried using a Büchi 190 labtop spray dryer using an inlet temperature of 74°C and an outlet temperature of 55°C. The flow rate was about 5.7 mL/min and the air atomization pressure was about 32 psi. The spray-dried powder product was collected in 66.4% yield, based on the combined weights of the insulin and DPPC employed.

Example 3

Preparation of Spray-dried Insulin:DPPC (10:90) Powder

A solution of DPPC was prepared at a concentration of 45 mg/mL in absolute ethanol. A powder of insulin particles prepared as described in Example 1 was added to the DPPC/ethanol solution at a concentration of 5 mg/mL. The insulin particles were further dispersed into the suspension by gentle sonication. While being gently agitated, this suspension was then spray dried using a Büchi 190 labtop spray dryer in a manner similar to that described in Example 2. The spray-dried powder product was collected in 65.9% yield.

Example 4

Preparation of Spray-dried Insulin:DPPC (90:10) Powder

A solution of DPPC was prepared at a concentration of 5 mg/mL in absolute ethanol. A powder of insulin particles prepared as described in Example 1 was added to the DPPC/ethanol solution at a concentration of 45 mg/mL. The insulin particles were further dispersed into the suspension by gentle sonication. While being gently agitated, this suspension was then spray dried using a Büchi 190 labtop

spray dryer in a manner similar to that described in Example 2. The spray-dried powder product was collected in 63.1% yield.

Example 5

Analyses of Spray-dried Powders

The insulin content of the powders prepared in Examples 2, 3 and 4, the spray-dried insulin particles used to prepare them as described in Example 1, and the starting zinc-insulin crystals was determined by HPLC analysis using a 15 cm reverse-phase Vydac (Hesperia, CA, USA) Protein and Peptide C18 column using as a mobile phase a solution of 25% acetonitrile and 75% of 0.2 M sodium sulfate buffer at pH 2.3.

The insulin purity in all test samples was determined by HPLC analysis using a 25 cm Vydac Protein and Peptide C18 column using an increasing acetonitrile gradient in 0.2 M sodium sulfate buffer at pH 2.3. The largest non-polymeric impurity in each of the samples tested was Asp(A21)-desamido-human insulin.

The polymer content of the insulin test samples was determined by size-exclusion HPLC using a 30 cm Waters (Milford, MA, USA) Protein-Pak 125 insulin assay-certified column using as a mobile phase a solution of 20% acetonitrile, 15% glacial acetic acid and 65% of a 1.0 mg/mL L-arginine solution.

The median diameter of the particles contained within the powders prepared in Examples 2, 3 and 4 was determined on a LS130 model Coulter counter (Beckman Coulter, Miami, FL, USA). The data from these analyses are displayed in Table 1 below.

Table 1

Analyses of zinc-insulin and spray-dried insulin powders

Sample	Insulin content (% by weight)	Percent Insulin Purity	Percent Polymers	Volume Median Particle Diameter (μm)
Zinc-insulin	90.1	99.2	0.08	nd
Insulin solid (Ex. 1) used in Ex. 2	72.4	98.9	0.12	nd
Insulin:DPPC (50:50) powder (Ex. 2)	40.2	98.7	0.18	3.69
Insulin solid (Ex. 1) used in Ex. 3	70.7	98.8	0.11	nd
Insulin:DPPC (10:90) powder (Ex. 3)	7.6	99.3	0.21	3.42
Insulin solid (Ex. 1) used in Ex. 4	74.2	99.0	0.14	nd
Insulin:DPPC (90:10) powder (Ex. 4)	67.9	98.9	0.14	3.94

nd=not determined

The data for Examples 2, 3 and 4 show that spray drying insulin particle suspensions in ethanol containing DPPC led to good recovery of powders of DPCC-coated insulin particles. The powders retained a high degree of insulin purity and contained very low levels of insulin polymers. This indicates the particles of the present invention may be successfully prepared by the double spray-drying process described herein. The process used in preparing these particles minimized or avoided conditions that lead to protein degradation or polymer formation.

The range of median sizes of the DPPC-coated insulin particles in the spray-dried powders of Examples 2, 3 and 4, 3.42 μm to 3.94 μm, is suitable for pulmonary administration to the deep lung.

Example 6

Insufflation of Powders in Rats

The powders of spray-dried particles generated in Examples 1-3 were tested by insufflation in 200-259 g male Fisher 344 rats. The animals were anesthetized using isoflurane until loss of reflexes to interdigital pinch was obtained.

The powders were combined with inhalation grade lactose employing geometric dilution. The lactose-diluted materials (10 mg) were placed into size 00 capsules and loaded into a Penn Century dosing device as described by Century, T. J., in U.S. Patent No. 5,570,686, issued 5 November 1996 and U.S. Patent No. 5,542,412, issued 6 August 1996.

The anesthetized animals were placed on a clear, 45° inclined platform. The metal cannula of the dosing device was inserted into the trachea of the rats. Verification of the placement of the cannula was conducted by gently palpating for the tracheal rings and checking for condensation of breath onto chilled dental mirrors. Dosing

was synchronized with the natural breathing pattern of the animals to minimize loss on inhalation.

Four different samples were administered to each of six animals. Sample 1 was a control sample of zinc-insulin crystals dissolved in dilute HCl, then adjusted to pH 7.5 with dilute NaOH. This sample was injected subcutaneously at a dose of 75 micrograms of insulin per kg of body weight. Sample 2 was a powder of spray-dried, uncoated insulin particles generated as described in Example 1 that was administered by insufflation at a dose of 500 micrograms of insulin per kg of body weight. Samples 3 and 4 were powders of spray-dried, DPPC-coated insulin particles prepared in a manner as described in Examples 2 and 3, respectively, that were administered to the rats by insufflation at doses of 1000 micrograms and 350 micrograms per kg of body weight, respectively, based on the insulin content of the powders.

Blood samples were obtained from each animal at various times for up to 12 hours and the serum glucose values were determined using standard procedures. The average percent of starting glucose values (n=6) and the standard error of the mean are listed in Table 2.

Table 2

Average percent of starting blood glucose values (± standard error of the mean) obtained after administration of Samples 1 to 4 to rats (n=6)

Time (hr.)	Sample 1	Sample 2	Sample 3	Sample 4
0	100	100	100	100
0.25	62.9 ± 18.5	65.2 ± 4.5	74.2 ± 8.7	65.0 ± 3.6
0.50	34.5 ± 4.0	49.0 ± 3.9	42.9 ± 6.0	35.3 ± 5.6
1	42.2 ± 3.4	51.5 ± 3.8	38.5 ± 2.5	32.6 ± 3.3
3	51.4 ± 4.1	64.0 ± 7.1	46.6 ± 2.0	33.2 ± 4.0
4	99.0 ± 4.2	50.6 ± 7.3	73.3 ± 8.2	26.7 ± 3.5
6	103.4 ± 8.1	96.4 ± 6.5	67.9 ± 6.5	39.3 ± 10.1
8	102.1 ± 4.7	99.9 ± 6.8	68.5 ± 9.7	39.8 ± 6.0
12	102.0 ± 9.6	87.8 ± 11.3	88.2 ± 2.8	75.7 ± 5.4

These data show that all three spray-dried, insulin-containing powders, Samples 2, 3 and 4, showed efficacy in lowering blood glucose when administered by insufflation to rats. Insufflation of the powders of insulin particles coated with DPPC (Samples 3 and 4) resulted in prolonged time action compared to insufflation of uncoated insulin particles (Sample 2).

Sample 4, the powder comprised of insulin particles coated with a much greater quantity of DPPC, by weight, compared to insulin, showed a significantly extended time action in the rats compared to the particles in which the weights of the insulin and the DPPC coating were comparable (Sample 3). The extended blood glucose lowering activity

was especially evident 4 to 8 hours after the powders were administered. These data clearly demonstrate that beneficial results may be obtained when a broad range of proportions of a therapeutic protein and a surfactant can be incorporated into respirable particles.

Example 7

Preparation of Micronized Insulin Particles

Zinc crystals of recombinant human insulin were added slowly to a Trost™ Gem-T model air impact pulverizer (Glen Mills, Inc., Clifton, New Jersey, USA) at an inlet nitrogen pressure of 80 psi and outlet nitrogen pressure of 100 psi. The jet milled micronized particles had a volume mean diameter of 7.5 μm as measured with a Coulter LS Particle Size Analyzer in aqueous suspension. More than half of the particles were less than or equal to 5 μm in size.

Example 8

Preparation of Spray-dried Insulin:DPPC (5:95) Powder

A solution of DPPC was prepared at a concentration of 47.5 mg/mL in absolute ethanol. A powder of insulin particles prepared as described in Example 1 was added to the DPPC/ethanol solution at a concentration of 2.5 mg/mL. The insulin particles were further dispersed into the suspension by gentle sonication. While being gently agitated, this suspension was then spray dried using a Büchi 190 labtop spray dryer in a manner similar to that described in Example 2. The spray-dried powder product was collected in 73.4% yield.

Example 9

Inhalation of Insulin Powders in Monkeys

Several insulin-containing powders were evaluated by inhalation in monkeys. Four adult cynomolgus monkeys, each weighing 3.4 to 4.2 kg, were restrained in specially designed chairs prior to inhalation administration. Aerosols of the insulin-containing powders were generated using a Vilnus dust generator operated at 10 liters per minute and which had an output of approximately 5.5 liters per minute. Total exhaust airflow was approximately 8 liters per minute. Pulmonary function parameters (tidal volume, frequency and minute volume) were measured during a 5 minute control period and a 5 minute exposure time.

Three different powders were administered to the monkeys. Sample 5 was a powder of micronized insulin particles prepared as described in Example 7. Sample 6 was spray-dried insulin:DPPC (10:90) powder prepared in a manner as described in Example 3. Sample 7 was spray-dried insulin:DPPC (5:95) powder prepared as described in Example 8. The targeted inhalation dose to the lung was 17.5 micrograms per kg of body weight, based on the insulin content of the powders.

Blood samples were obtained at various times up to 24 hours post-dose and the serum glucose values were determined using standard procedures. The average percent of starting glucose values and the standard error of the mean are listed in Table 3.

Table 3

Average percent of starting blood glucose values (± standard error of the mean) obtained after inhalation of Samples 5 to 7 in monkeys

Time (hours)	Sample 5 (n=6)	Sample 6 (n=8)	Sample 7 (n=2)
0	100	100	100
0.5	52.9 ± 8.3	67.4 ± 6.9	58.8 ± 7.4
1	48.6 ± 8.0	51.3 ± 9.3	43.5 ± 12.8
2	47.1 ± 7.9	60.2 ± 7.3	41.0 ± 10.0
4	52.3 ± 7.5	70.3 ± 6.3	45.0 ± 13.8
6	63.7 ± 5.6	85.5 ± 4.4	53.7 ± 8.2
8	69.1 ± 5.2	71.9 ± 4.7	58.1 ± 14.5
10	70.6 ± 5.9	65.5 ± 4.4	57.2 ± 13.3
12	70.6 ± 6.9	69.3 ± 5.0	46.4 ± 14.9
24	83.3 ± 5.6	88.6 ± 8.3	85.1 ± 13.4

These data demonstrate that each powder had efficacy in lowering blood glucose when administered by inhalation to monkeys.

Example 10

Inhalation of Insulin Powders in Dogs

Two insulin-containing powders were evaluated by administration by inhalation to dogs. Four adult female beagles, each weighing 10.8 to 14.1 kg, were fasted approximately 12 hours prior to dosing. The dogs were exposed to the various aerosols while standing in a

restraint sling. One layer of a 0.03 inch latex sheet was placed around the animals' neck to form a non-restrictive airtight seal. A custom built 11-liter head dome was placed over the dogs' heads and secured to the restraint device. The total air flow rate through the dome was about 8 liters per minute.

Aerosols of the insulin-containing powders were generated using a Vilnus dust generator with an input of approximately 5.5 liters per minute. Total output from the generator flowed directly into the head dome. One gravimetric sample was collected during each 5 to 15 minute exposure which proceeded at a flow rate of 1 liter per minute.

Sample 8 was a powder of micronized insulin particles prepared as described in Example 7. Sample 9 was spray-dried insulin:DPPC (10:90) powder prepared in a manner as described in Example 3. The targeted inhalation dose to the lung was 17.5 micrograms per kg of body weight, based on the insulin content of the powders.

Blood samples were obtained at various times up to 24 hours post-dose and the serum glucose values were determined using standard procedures. The average percent of starting glucose values and the standard error of the mean are listed in Table 4.

Table 4

Average percent of starting blood glucose values (± standard error of the mean) obtained after inhalation of Samples 8 and 9 in dogs

Time (hours)	Sample 8 (n=4)	Sample 9 (n=5)
0	100	100
0.5	47.6 ± 6.9	59.4 ± 12.8
1	37.9 ± 2.9	35.9 ± 3.5
2	63.4 ± 8.6	28.3 ± 1.7
4	86.7 ± 17.0	26.8 ± 1.9
6	108.2 ± 6.6	48.5 ± 4.7
8	nd	70.2 ± 11.8
10	nd	93.0 ± 17.1
12	nd	101.9 ± 14.9
16	nd	105.5 ± 8.6
24	nd	100.3 ± 9.7

nd = not determined

These data demonstrate that each powder had efficacy in lowering blood glucose when administered by inhalation to dogs. Sample 9, the spray-dried insulin:DPPC (10:90) powder, showed a prolonged time action compared to the micronized insulin powder (Sample 8) as evidenced by the much lower blood glucose levels that were measured 2, 4 and 6 hours after the powders were administered.

Example 11

Preparation of Spray-dried Insulin-DSPC(10:90) Powder

A solution of DSPC (distearoylphosphatidylcholine, Avanti Polar Lipids, Lot #180PC-101) was prepared at a concentration of 45 mg/mL in absolute ethanol. A powder of insulin particles prepared as described in Example 1 was added to the DSPC/ethanol solution at a concentration of 5 mg/mL. The insulin particles were further dispersed into the suspension by gentle intermittent sonication over two minutes. While being gently agitated, this suspension was then spray dried using a Büchi 190 labtop spray dryer in a manner similar to that described in Example 2. The spray-dried powder product was collected in 61.3% yield.

Example 12

Preparation of Spray-dried Val(8)-GLP-1(7-37)OH Solid

Val(8)-GLP-1(7-37)OH, at 98.7% purity by HPLC, was dissolved at 10 mg/mL in a solution of 0.1 M sodium citrate pH 2.0 buffer and filtered through a 0.45 μm filter. The filtered solution was kept cool and quiescent prior to spray drying. The Val(8)-GLP-1(7-37)OH solution was spray dried using a Büchi 190 labtop spray dryer with the inlet temperature set at 81°C and the outlet temperature set at 60°C. The flow rate was about 4.0 mL/min and the air atomization pressure was about 32 psi. The resulting white powder of Val(8)-GLP-1(7-37)OH particles was scraped from the glass collection jar with a spatula giving a yield of 82% by weight. HPLC analysis using a 25 cm reversed-phase Zorbax (Mac-Mod, Chadds Ford, Pennsylvania, USA) 300 SB C8 column and an increasing gradient of acetonitrile with 0.1% trifluoroacetic acid in water revealed a Val(8)-GLP-1(7-37)OH purity of 98.8%.

Example 13

Preparation of Spray-dried

Val(8)-GLP-1(7-37)OH:DPPC (10:90) Powder

A solution of DPPC was prepared at a concentration of 45 mg/mL in absolute ethanol. A powder of Val(8)-GLP-1(7-37)OH particles prepared as described in Example 12 was added to the DPPC/ethanol solution at a concentration of 5 mg/mL. The Val(8)-GLP-1(7-37)OH particles were further dispersed into the suspension by gentle sonication for at least one minute. While being gently agitated, this suspension was then spray dried using a Büchi 190 labtop spray dryer using an inlet temperature of 74°C and an outlet temperature of 55°C. The flow rate was about 6.7 mL/min and the air atomization pressure was about 32 psi. The white spray-dried powder product was collected in a glass amber jar in 34.6% yield and an HPLC purity of 80.8%.

Analysis of the powder by scanning electron microscopy revealed fine particles between 1 μm and 5 μm in diameter. A larger volume median particle diameter of 7.7 μm was measured in the Coulter counter, however, probably due to particle aggregation.

WE CLAIM:

1. A particle suitable for pulmonary delivery to the deep lung of a patient in need thereof, comprising a therapeutic protein core and a surfactant coating.

2. The particle according to claim 1, wherein the particle is nonporous.

3. The particle according to claim 1, wherein the surfactant is a phospholipid.

4. The particle according to claim 3, wherein the surfactant is DPPC.

5. The particle according to claim 1, wherein the therapeutic protein is a hormone, an antibody or an enzyme.

6. The particle according to claim 5, wherein the therapeutic protein is a hormone.

7. The particle according to claim 6, wherein the therapeutic protein is a native form of insulin, GLP-1, exendin, PTH, EPO, FSH, leptin, growth hormone, or an analog or derivative thereof.

8. The particle according to claim 7, wherein the therapeutic protein is a native form of insulin, an insulin analog or a derivative thereof.

9. The particle according to claim 8, wherein the therapeutic protein is human insulin.

10. The particle according to claim 7, wherein the therapeutic protein is GLP-1, a GLP-1 analog or a derivative thereof.

11. The particle according to claim 10, wherein the therapeutic protein is Val(8)-GLP-1(7-37)OH.

12. The particle according to any one of claims 1 to 11, for use in treating a disease or disorder.

13. The particle according to claim 12, for use in treating diabetes or hyperglycemia.

14. The use of the particle according to any one of claims 1 to 11, to prepare a medicament for the treatment of a disease or disorder by pulmonary administration.

15. The use according to claim 14, wherein the medicament is for the treatment of diabetes or hyperglycemia.

16. A method of treating a disease or disorder comprising, administering to a patient in need thereof, the particle according to any one of claims 1 to 11, such that the particle is deposited in the deep lung of the patient.

17. The method according to claim 16, wherein the disease or disorder being treated is diabetes or hyperglycemia.

18. A powder, suitable for pulmonary delivery, comprising the particle according to any one of claims 1 to 11.

19. The powder according to claim 18, for use in treating a disease or disorder.

20. The powder according to claim 19, for use in treating diabetes or hyperglycemia.

21. The use of the powder according to claim 18, to prepare a medicament for the treatment of a disease or disorder by pulmonary administration.

22. The use according to claim 21, wherein the medicament is for the treatment of diabetes or hyperglycemia.

23. A method of treating a disease or disorder comprising, administering to a patient in need thereof, an effective amount of the powder according to claim 18, such that the powder is deposited in the deep lung of the patient.

24. The method according to claim 23, wherein the disease or disorder being treated is diabetes or hyperglycemia.

25. A process for preparing the powder according to claim 18, comprising:

a) preparing a suspension composition comprising particles of a therapeutic protein, a liquid carrier, and a surfactant;

b) spray drying the suspension composition of step a) to produce a powder; and

c) recovering the powder produced in step b).

26. The process according to claim 25, wherein the surfactant in the suspension composition is a phospholipid.

27. The process according to claim 26, wherein the surfactant is DPPC.

28. The process according to any one of claims 25 to 27, wherein the liquid carrier is an organic solvent.

29. The process according to claim 28, wherein the organic solvent is ethanol.

30. The process according to any one of claims 25 to 29, wherein the particles of the therapeutic protein are prepared by:

a) preparing a solution comprising the therapeutic protein;

b) spray drying the protein solution of step a) to produce protein particles; and

c) recovering the protein particles produced in step b);

31. The process according to any one of claims 25 to 29, wherein the particles of the therapeutic protein are prepared by micronization, milling, freeze drying, jet milling or microcrystallization.

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(54) Title: PROTEIN POWDER FOR PULMONARY DELIVERY

(57) Abstract: This invention provides particles of a therapeutic protein core coated with a surfactant that are suitable for pulmonary delivery to the deep lung of a patient in need thereof. Spray drying processes for preparing powders of the core protein particles and powders of the respirable, surfactant-coated, therapeutic protein particles are provided.

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Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, CHEM ABS Data

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[X] Further documents are listed in the continuation of box C. [X] Patent family members are listed in annex.

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